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Temporal Order of RNA-Processing Reactions in Trypanosomes: Rapid *trans* Splicing Precedes Polyadenylation of Newly Synthesized Tubulin Transcripts

ELISABETTA ULLU,[1,2] KEITH R. MATTHEWS,[1]† AND CHRISTIAN TSCHUDI[1]*

Departments of Internal Medicine[1] and Cell Biology,[2] Yale MacArthur Center for Molecular Parasitology, Yale University School of Medicine, 333 Cedar Street, New Haven, Connecticut 06510-8056

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Many trypanosome genes are expressed as part of large polycistronic transcription units. This finding suggests that regulation of mRNA biogenesis may emphasize RNA-processing reactions more so than in other organisms. This study was undertaken to understand the temporal order of two RNA-processing reactions, *trans* splicing and polyadenylation, in the maturation of trypanosome mRNAs in vivo. Kinetic studies revealed rapid *trans* splicing of α-tubulin, β-tubulin, and actin pre-mRNAs within 1 to 2 min after synthesis of the 3′ splice site. Furthermore, following blockage of pre-mRNA synthesis, newly synthesized spliced leader RNA cannot be used for *trans* splicing, suggesting that trypanosomes do not accumulate substantial amounts of pre-mRNA which can provide splice acceptor sites. Thus, *trans* splicing is cotranscriptional. In addition, we show that *trans* splicing precedes polyadenylation in the processing of trypanosome tubulin pre-mRNAs.

The biosynthesis of mRNA in *Trypanosoma brucei* differs in at least two aspects from that in other eukaryotic organisms. First, it appears that protein-coding genes are organized in polycistronic transcription units giving rise to RNA primary transcripts that contain more than one mRNA coding region in the same topological arrangement as in the chromosome (13, 20, 24, 31). Second, the 5′ ends of all known trypanosome mRNAs are generated by an RNA-processing reaction, namely, *trans* splicing, rather than by transcription initiation (15).

trans splicing in *T. brucei* entails the joining of the spliced leader (SL) RNA, a small capped RNA of approximately 140 nucleotides (nt), and pre-mRNA by transferring the first 39 nt of the SL RNA (the SL or mini-exon sequence) to the 5′ end of all mRNAs. It has become clear over the last several years that from a mechanistic point of view, *trans* splicing is closely related to *cis* splicing of intervening sequences (1). For example, molecules that are structurally analogous to the *cis*-splicing intron-exon lariat intermediates were identified in trypanosome cells; these molecules were linear Y-branched molecules consisting of the SL intron joined to high-molecular-weight RNA via a 2′-5′ phosphodiester bond (17, 21, 28). Also, the requirement of U2, U4, and U6 small nuclear RNAs for *trans* splicing in trypanosomes (32) emphasizes the similarities between the cofactors involved in *cis* and *trans* splicing.

What is the function of *trans* splicing in trypanosomes? *trans* splicing might provide a means to resolve complex polycistronic pre-mRNAs into monocistronic mRNAs. In addition, the joining of the SL sequence to the 5′ end of mRNAs fulfills the important function of providing the mRNA with a cap structure. In other systems, cap structures can play a role in mRNA translation initiation (3, 8, 26) and may protect mRNAs against degradation (10, 27). Indeed, trypanosome α-tubulin pre-mRNA is rapidly degraded in permeable cells when *trans* splicing is inhibited (32).

The suggestion that long polycistronic pre-mRNAs are precursors to mature mRNAs in trypanosomes raises the question of whether these transcripts are synthesized in toto or whether maturation takes place on nascent transcripts. In either case, pre-mRNAs must be processed at the 5′ end by *trans* splicing and at the 3′ end by the addition of a poly(A) tail. In this work, we investigated the timing and the efficiency of these RNA-processing reactions in permeable trypanosome cells. We provide evidence that unprocessed pre-mRNAs do not accumulate, most likely because pre-mRNAs are processed as they are synthesized. Moreover, for the tubulin gene transcripts, it appears that polyadenylation does not occur if we block *trans* splicing.

MATERIALS AND METHODS

Plasmid constructions. cDNA clones covering the mature 5′ ends of various trypanosome mRNAs were constructed according to the following procedure outlined for the β-tubulin mRNA. Total RNA from procyclic trypanosome forms derived from *T. brucei rhodesiense* YTat 1.1 (31) was primed with a DNA oligonucleotide complementary to nt 2 to 21 of the translated region of β-tubulin (14) to generate a cDNA containing the complement of the SL at the very 3′ end. Double-stranded cDNA was synthesized with an SL sense oligonucleotide and amplified with the polymerase chain reaction, using the SL- and β-tubulin-specific primers. The amplified cDNA was cloned into plasmid T3T7*lac* (Boehringer Mannheim), and the DNA insert was verified by DNA sequencing. The following gene-specific primers were used to construct the corresponding cDNA clones: α-TUB-164, nt 2 to 21 of α-tubulin (14, 32); α-TUB-275, nt 114 to 130 of α-tubulin (14); α-TUB-555, nt 391 to 408 of α-tubulin (14); β-TUB-119, nt 2 to 21 of β-tubulin (14); β-TUB-227, nt 112 to 129 of β-tubulin (14); actin-234, nt 123 to 140 of actin (4); and PARP-135, nt 44 to 65 of procyclic acidic repetitive protein (PARP) (6, 23, 24). A DNA fragment (probe 19) covering the poly(A) addition sites of the α-tubulin gene was prepared by

* Corresponding author.
† Present address: Department of Biochemistry and Molecular Biology, University of Manchester, Manchester, United Kingdom M13 9PT.

the polymerase chain reaction, using one oligonucleotide complementary to nt 1071 to 1090 of the α-tubulin translated region (14) and the other complementary to nt 143 to 159 downstream of the α-tubulin termination codon (14).

Labeling of newly synthesized RNA in permeable trypanosome cells. Procyclic trypanosome cells were permeabilized with L-α-lysophosphatidylcholine and palmitoyl (lysolecithin), and RNA was labeled with [α-^{32}P]UTP as described previously (32). For time course experiments, cells were labeled under standard conditions (32), and the reaction was stopped at the indicated time points by mixing an aliquot of the incubation mixture with an equal volume of a solution containing 2 mg of proteinase K per ml, 50 mM EDTA, and 2% sodium dodecyl sulfate (SDS). After incubation at 55°C for 30 min, nucleic acids were precipitated with ethanol and treated with RNase-free DNase I (100 μg/ml, final concentration).

RNase mapping. The RNase mapping experiments shown in Fig. 1A and 2 were carried out with biotinylated antisense RNA probes, and the hybrids were enriched by one cycle of selection with streptavidin-agarose essentially as described previously (31). The newly synthesized SL RNA was assayed with an antisense RNA probe complementary to nt 7 to 128 of the SL RNA (35). The probe was not biotinylated, and the protected fragments were displayed without further selection (Fig. 1B). The RNase-protected fragments shown in Fig. 3 to 5 were obtained as follows. Antisense RNA probes (without biotin) were hybridized to newly synthesized ^{32}P-labeled transcripts in 20 μl of hybridization buffer containing 80% formamide, 400 mM NaCl, 30 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES; pH 6.4), and 1 mM EDTA at 56°C for 18 to 24 h. The samples were treated with RNase by adding 200 μl of 300 mM NaCl–20 mM Tris (pH 7.5)–5 mM EDTA-5 U of RNase T_1 (Calbiochem) per ml–20 μg of RNase A (Calbiochem) per ml. After 1 h at 4°C, the samples were treated with 200 μg of proteinase K per ml in the presence of 0.5% SDS for 30 min at 55°C, and nucleic acids were precipitated with ethanol. RNAs were resuspended in hybridization buffer, denatured at 80°C for 10 min, rehybridized at 56°C for 18 to 24 h, and digested with RNase as described above. No additional probe was added to the second round of hybridization.

RESULTS

De novo synthesis of pre-mRNA is required to sustain active *trans* splicing of the SL RNA. To study the machinery that generates mature mRNAs in trypanosomes, we permeabilize cultured procyclic cells of *T. brucei* with the detergent lysolecithin (34). The resulting cell preparation efficiently incorporates radiolabeled nucleoside triphosphates into newly synthesized RNA and maintains the ability to process pre-mRNAs by *trans* splicing with the SL RNA (32, 34) and by polyadenylation (see below).

In the first set of experiments, we examined the effect on utilization in *trans* splicing of the SL RNA when we selectively blocked the synthesis of pre-mRNA by using the transcription inhibitor α-amanitin. In Fig. 1A, we monitored the accumulation and *trans* splicing of α-tubulin RNA in the presence of increasing concentrations of α-amanitin by RNase mapping with an RNA probe complementary to the first 164 nt of mature α-tubulin mRNA. Control cells (lane 1) revealed protected RNA fragments diagnostic of *trans*-spliced and unspliced α-tubulin RNAs (34). As expected from our previous results (34), incubation of permeable trypanosome cells with 1 μg of α-amanitin per ml (lane 2)

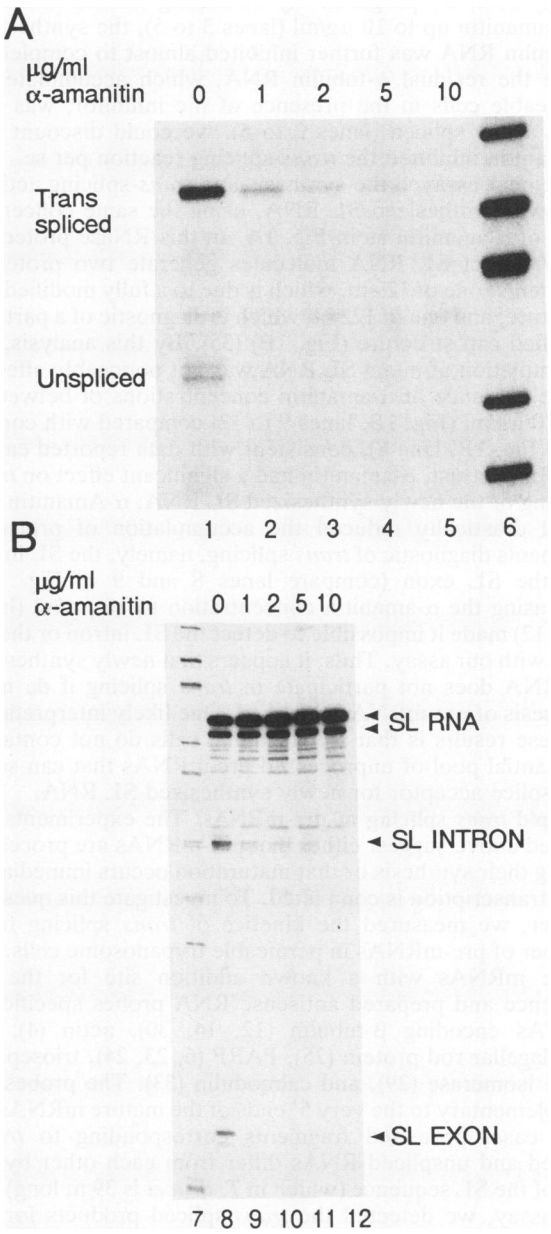

FIG. 1. Analysis of newly synthesized α-tubulin and SL RNAs in the presence of increasing concentrations of α-amanitin. Permeable procyclic trypanosome cells were preincubated without (lanes 1 and 8) or with different concentrations of α-amanitin for 1 min at room temperature; then [α-^{32}P]UTP was added, and RNA was synthesized for 10 min at 30°C. (A) RNase protection with α-TUB-164; (B) RNase protection analysis of newly synthesized SL RNA. Full-length SL RNA gives two protected fragments. The longer fragment of 129 nt originates from SL RNA with a modified 5′ end, whereas SL RNA with a partially modified cap structure gives rise to a shorter protected fragment of 122 nt (34). This assay also displays protected fragments characteristic of *trans* splicing of newly synthesized SL RNA (33). The 90-nt fragment (SL intron) derives from the linear and branched forms of the SL intron, and the 40-nt fragment (SL exon) reveals the SL exon or SL sequence which has been joined by *trans* splicing to a variety of mRNAs. ^{32}P-labeled *Msp*I-digested pBR322 was used as a molecular weight marker and run in lanes 6 and 7.

reduced the accumulation of α-tubulin RNA by approximately 80%. By progressively increasing the concentration of α-amanitin up to 10 μg/ml (lanes 3 to 5), the synthesis of α-tubulin RNA was further inhibited almost to completion. Since the residual α-tubulin RNA, which accumulated in permeable cells in the presence of the inhibitor, was correctly *trans* spliced (lanes 2 to 5), we could discount that α-amanitin inhibited the *trans*-splicing reaction per se.

We next assayed the synthesis and *trans*-splicing activity of newly synthesized SL RNA, using the same concentrations of α-amanitin as in Fig. 1A. In this RNase protection assay, intact SL RNA molecules generate two protected fragments: one of 129 nt, which is due to a fully modified cap structure, and one of 122 nt, which is diagnostic of a partially modified cap structure (Fig. 1B) (35). By this analysis, the accumulation of intact SL RNA was not noticeably affected in the presence of α-amanitin concentrations of between 1 and 10 μg/ml (Fig. 1B, lanes 9 to 12) compared with control cells (Fig. 1B, lane 8), consistent with data reported earlier (34). In contrast, α-amanitin had a significant effect on *trans* splicing of the newly synthesized SL RNA. α-Amanitin at 1 μg/ml drastically reduced the accumulation of protected fragments diagnostic of *trans* splicing, namely, the SL intron and the SL exon (compare lanes 8 and 9 in Fig. 1B). Increasing the α-amanitin concentration to 10 μg/ml (lanes 10 to 12) made it impossible to detect the SL intron or the SL exon with our assay. Thus, it appears that newly synthesized SL RNA does not participate in *trans* splicing if de novo synthesis of pre-mRNA is blocked. One likely interpretation of these results is that trypanosome cells do not contain a substantial pool of unprocessed pre-mRNAs that can serve as a splice acceptor for newly synthesized SL RNA.

Rapid *trans* splicing of pre-mRNAs. The experiments described above suggest either that pre-mRNAs are processed during their synthesis or that maturation occurs immediately after transcription is completed. To investigate this question further, we measured the kinetics of *trans* splicing for a number of pre-mRNAs in permeable trypanosome cells. We chose mRNAs with a known addition site for the SL sequence and prepared antisense RNA probes specific for mRNAs encoding β-tubulin (12, 14, 30), actin (4), the paraflagellar rod protein (25), PARP (6, 23, 24), triosephosphate isomerase (29), and calmodulin (33). The probes are complementary to the very 5′ ends of the mature mRNAs. In each case, protected fragments corresponding to *trans*-spliced and unspliced RNAs differ from each other by the size of the SL sequence (which in *T. brucei* is 39 nt long). By this assay, we detected the *trans*-spliced products for the β-tubulin, PARP, and actin (Fig. 2) as well as for the mRNAs encoding the paraflagellar rod protein and triosephosphate isomerase (data not shown). However, we could never detect the *trans*-spliced product of the calmodulin mRNA by this technique (data not shown).

In these initial experiments, we found that the amount of *trans*-spliced RNA detected for PARP, the paraflagellar rod protein, and triosephosphate isomerase transcripts was too low to be amenable to a kinetic study (data not shown). We therefore concentrated on the α-tubulin, β-tubulin, and actin pre-mRNAs. To measure the *trans*-splicing kinetics of these pre-mRNAs, [α-^{32}P]UTP-labeled newly synthesized RNA was isolated from permeable cells at various time points after the addition of the label and assayed by RNase mapping (Fig. 3). All three unspliced RNAs were clearly detected 30 s after the addition of the label and continued to increase over the time period of the experiment. For the α-tubulin (Fig. 3A and 4A) and β-tubulin (Fig. 3B) pre-mRNAs, the

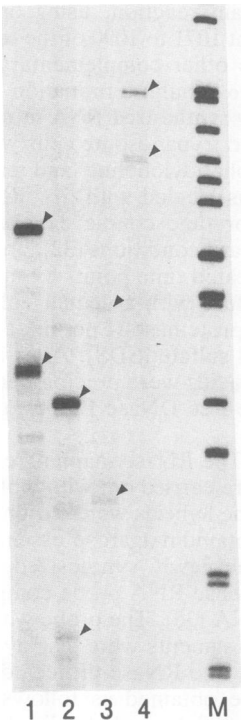

FIG. 2. Detection of the *trans*-spliced 5′ end of different transcripts. ^{32}P-labeled RNA was synthesized in permeable cells for 10 min, and RNase protection was performed with probes complementary to the mature 5′ end of α-tubulin RNA (lane 1), β-tubulin RNA (lane 2), PARP RNA (lane 3), and actin RNA (lane 4). Protected fragments corresponding to *trans*-spliced (upper) and unspliced (lower) RNAs are indicated by arrowheads. ^{32}P-labeled *Msp*I-digested pBR322 was used as molecular weight marker and run in lane M.

appearance of the *trans*-spliced product was evident after 90 s. *trans*-spliced RNA of the actin pre-mRNA (Fig. 3C) was barely visible after 90 s but was clearly detectable after 2 min of incubation.

To test whether our ability to detect *trans*-spliced products at time points earlier than 90 s was impaired by the sensitivity of our assay, we generated an α-tubulin antisense RNA probe (α-TUB-555; Fig. 4B) which was twice as long as the probe used in Fig. 3A. Next, we repeated the measurements of the α-tubulin pre-mRNA by using the short (275-nt) and long (555-nt) probes for RNase mapping with aliquots from the same time course (Fig. 4). The results of this analysis demonstrated that when the longer antisense RNA probe was used, the apparent lag period for detection of labeled *trans*-spliced α-tubulin RNA was shortened to 60 s, presumably as a result of the increased sensitivity of the assay. The actual lag period may be even shorter, but obtaining an accurate measurement would require the use of probes longer than 555 nt. However, doing so causes an experimental problem because it will be difficult to separate *trans*-spliced and unspliced protected RNA fragments longer than 555 nt which differ by only 39 nt.

***trans* splicing precedes polyadenylation.** In the last set of experiments, we wished to determine the order of two RNA-processing reactions, *trans* splicing and polyadenylation, in the maturation of tubulin mRNAs in trypanosomes. The data shown in Fig. 5 were obtained for the α-tubulin

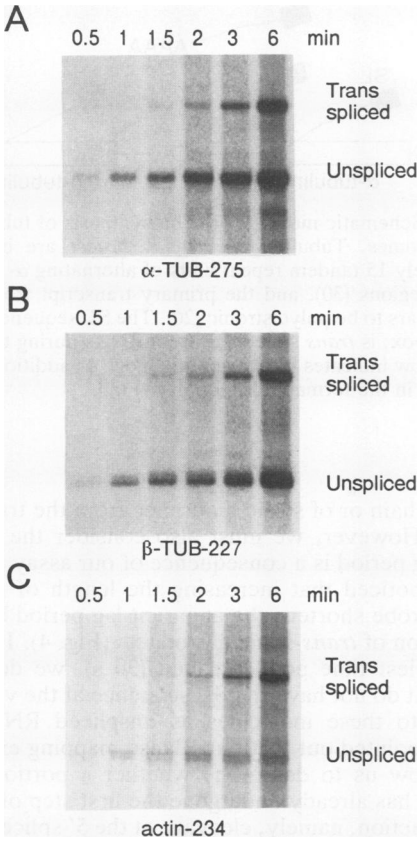

FIG. 3. Kinetic analysis of *trans* splicing. RNA labeling in permeable cells was initiated by the addition of [α-^{32}P]UTP, aliquots were removed at the indicated time points, and RNase mapping was performed with three different antisense RNA probes. (A) α-Tubulin transcripts assayed with α-TUB-275; (B) β-tubulin RNAs identified with β-TUB-227; (C) actin transcripts mapped with actin-234. In each case, the positions of the *trans*-spliced and unspliced RNAs are indicated.

transcripts, but essentially similar results were obtained for the processing of the β-tubulin transcripts. We first fractionated total RNA on oligo(dT)-cellulose and then assayed the bound and unbound RNA for *trans*-spliced and unspliced α-tubulin RNAs by RNase mapping (Fig. 5A). Unspliced α-tubulin RNA is almost exclusively recovered in the poly(A)$^-$ fraction (lane 2), suggesting that the majority of unspliced RNA is not polyadenylated. The *trans*-spliced RNA, on the other hand, partitions with approximately equal amounts in the poly(A)$^+$ and poly(A)$^-$ fractions (lanes 2 and 3), indicating that a portion of the tubulin transcripts has been *trans* spliced but has not yet acquired a poly(A) tail long enough to bind to oligo(dT)-cellulose.

We next examined whether addition of a poly(A) tail can occur in the absence of *trans* splicing. For this study, we inhibited *trans* splicing by destroying the U2 small nuclear RNA with complementary DNA oligonucleotides and RNase H (32) and assayed *trans*-spliced and unspliced tubulin transcripts in the poly(A)$^+$ and poly(A)$^-$ fractions (Fig. 5, lanes 5 and 6). With this treatment, the appearance of *trans*-spliced α-tubulin RNA is greatly reduced (compare lanes 1 and 4), and the majority of tubulin transcripts are unspliced and fractionate in the poly(A)$^-$ fraction (lane 5). Careful inspection of the original autoradiograph, but less

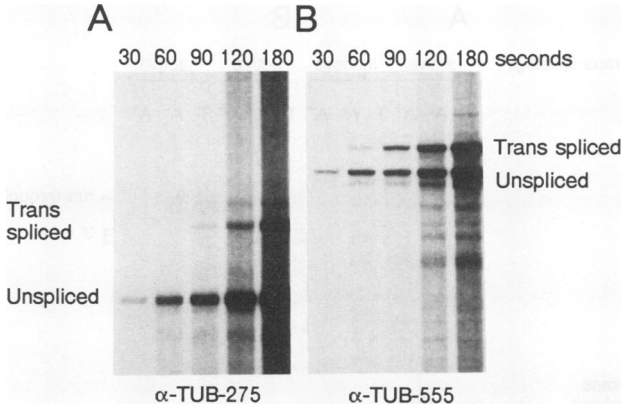

FIG. 4. *trans*-splicing kinetics of the α-tubulin transcripts. A time course of RNA synthesis was assayed for the appearance of *trans*-spliced α-tubulin RNA. The RNase mapping probes were 275 (A) and 555 (B) nt long.

visible in the reproduction shown in Fig. 5, revealed small amounts of unspliced RNA in the poly(A)$^+$ fraction (lanes 3 and 6), suggesting that a minute amount of polyadenylation can occur on unspliced RNA. Although this possibility exists, we find it more likely that this result is due to nonspecific sticking to oligo(dT)-cellulose.

To confirm these results, we directly assayed polyadenylation of α-tubulin transcripts by subjecting RNA from the fractions shown in Fig. 5A to RNase mapping with a probe specific for 3′ ends (Fig. 5B). Since α-tubulin transcripts are polyadenylated at three different sites with almost equal efficiency (19), we used as a probe an antisense RNA which spans all three sites. Therefore, transcripts without mature 3′ ends give rise to full protection of the probe (445 nt; labeled run-through in Fig. 5B), and these transcripts partition in the poly(A)$^-$ fraction (lane 8). Transcripts with mature 3′ ends will be cleaved at the poly(A) addition sites and generate three major protected fragments of 417, 393, and 389 nt (lane 7). That these transcripts are indeed polyadenylated is supported by their binding to oligo(dT)-cellulose (lane 9). An array of bands below the fragments corresponding to mature 3′ ends was consistently detected. The origin of these bands is presently unclear, but sequence heterogeneity in the 3′ untranslated region of the α-tubulin transcripts or breathing of the AT-rich hybrids could generate these RNase digestion products. Interestingly, under conditions in which *trans* splicing is inhibited (lanes 10 to 12), the majority of the α-tubulin transcripts are not processed at the 3′ end and are not polyadenylated (lane 11). Thus, conditions which block *trans* splicing also lead to inhibition of 3′ end formation. Taken together with our finding that approximately half of the *trans*-spliced α-tubulin RNA is not polyadenylated, these data suggest that *trans* splicing of the α-tubulin RNA precedes polyadenylation.

DISCUSSION

Kinetic studies suggest that 5′ end processing of pre-mRNAs by *trans* splicing with the SL RNA must take place relatively rapidly, since the half-life of the SL RNA is approximately 4 to 6 min (16, 17). In addition, in permeable cells, the products of *trans* splicing of newly synthesized SL RNA, the SL intron and SL exon, appear 3 to 4 min after the start of transcription (32; unpublished data). In the studies

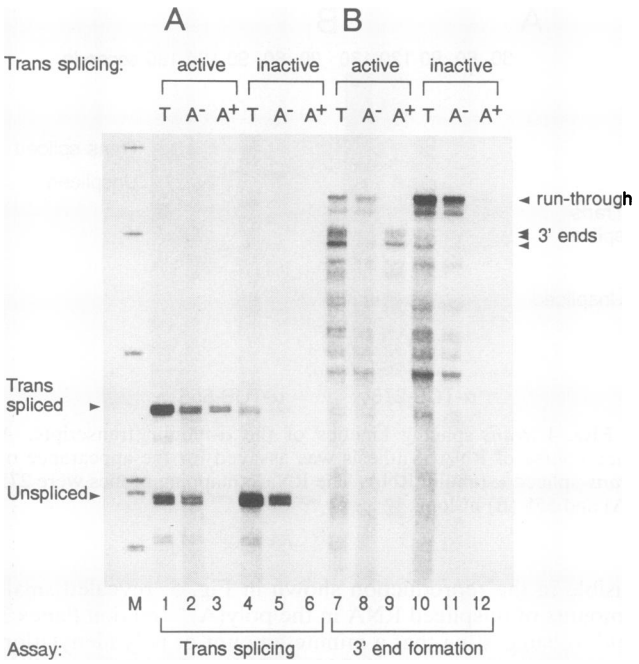

FIG. 5. Evidence that inhibition of *trans* splicing blocks 3′ end formation. Control cells (lanes 1 to 3 and 7 to 9) and cells treated with oligonucleotide U2SSC to inhibit *trans* splicing (lanes 4 to 6 and 10 to 12) were incubated in transcription cocktail, and ^{32}P-labeled RNA was synthesized for 10 min. One half of the RNA was fractionated on oligo(dT)-cellulose, and aliquots of unfractionated RNA (T), poly(A)$^-$ RNA (A$^-$), and poly(A)$^+$ RNA (A$^+$) were analyzed by RNase mapping. (A) *trans* splicing of α-tubulin transcripts monitored with α-TUB-275. The positions of the *trans*-spliced and unspliced fragments are indicated. (B) 3′ end formation of α-tubulin transcripts assayed with probe 19. Run-through, full protection of the probe (these fragments correspond to α-tubulin transcripts which have not been processed at the poly(A) addition sites); 3′ ends, protected fragments generated by α-tubulin transcripts which have been processed by the addition of a poly(A) tail.

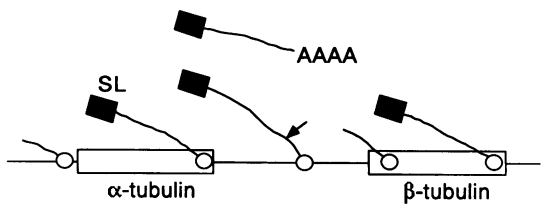

FIG. 6. Schematic model for the biosynthesis of tubulin mRNA in trypanosomes. Tubulin genes in *T. brucei* are organized in approximately 15 tandem repeat units of alternating α- and β-tubulin-coding regions (30), and the primary transcript from this gene cluster appears to be polycistronic (20). The SL sequence, indicated by a black box, is *trans* spliced to pre-mRNAs during their synthesis. The arrow indicates cleavage at the poly(A) addition site which then results in the formation of a poly(A) tail.

presented here, RNase mapping of ^{32}P-labeled newly synthesized transcripts revealed that α-tubulin *trans*-spliced products are detectable 1 min after the addition of the label. Assuming that the rate of elongation by RNA polymerase II in permeable trypanosome cells is similar to that of other eukaryotic RNA polymerases (20 to 25 nt/s [7]), approximately 1.2 to 1.5 kb of RNA is synthesized during the labeling period. The size of the α-tubulin mRNA body (from the 3′ splice site to the polyadenylation sites) is 1.8 kb (12, 14, 30), suggesting that *trans* splicing takes place just before or soon after completion of the synthesis of the α-tubulin mRNA body. However, tubulin genes in *T. brucei* are organized in approximately 15 tandem repeat units of alternating α- and β-tubulin-coding regions (30), and the primary transcript from this gene cluster appears to be polycistronic (20). Our results are therefore consistent with a model that polycistronic transcripts are not synthesized in toto but that processing by *trans* splicing takes place cotranscriptionally (Fig. 6).

The observed lag period of 60 s for the detection of *trans*-spliced products could reflect the time required for assembly of a functional *trans* spliceosome. Alternatively, the growing RNA chain might have to reach a certain length before the *trans*-splicing components can assemble on the pre-mRNA, perhaps as a result of folding requirements of the RNA chain or of steric hindrance from the transcription complex. However, we must also consider the possibility that the lag period is a consequence of our assay procedure. We have noticed that increasing the length of the RNase mapping probe shortens the apparent lag period by improving detection of *trans*-spliced products (Fig. 4). In addition, at the earliest time point assayed (30 s), we detect RNA species that do not have the SL sequence at the very 5′ end. We refer to these molecules as unspliced RNAs, but it should be pointed out that our RNase mapping experiments do not allow us to determine whether a portion of these transcripts has already undergone the first step of the *trans*-splicing reaction, namely, cleavage at the 5′ splice site of the SL RNA followed by joining of the 5′ end of the intron to the branch site upstream from the 3′ splice site of the pre-mRNA.

The analysis of *trans* splicing and polyadenylation of tubulin transcripts revealed two important aspects (Fig. 5). First, if we inhibit *trans* splicing, we also block the addition of a poly(A) tail, implying that *trans* splicing and polyadenylation are coupled processes. Indeed, functional studies in vivo point to the use of common signals by the polyadenylation and *trans*-splicing machineries (unpublished data). Second, chromatography on oligo(dT)-cellulose showed that approximately 50% of the *trans*-spliced tubulin transcripts are not polyadenylated. This observation allows us to specify the order of the two RNA-processing reactions. The fact that a fraction of already *trans*-spliced tubulin transcripts does not contain a poly(A) tail would favor the model that *trans* splicing precedes polyadenylation (Fig. 6). Our results differ from those reported by Huang and van der Ploeg (11). Analysis of *hsp70* transcripts in isolated nuclei suggested the possibility of cotranscriptional cleavage at the poly(A) addition site but revealed no evidence for cotranscriptional *trans* splicing. Although it remains to be determined whether this apparent discrepancy reflects the use of different systems (permeable cells versus isolated nuclei), both conclusions are compatible with published data on the processing of primary transcripts. Originally, experiments with simian virus 40, adenovirus, and the calcitonin/calcitonin gene-related peptide gene suggested that RNA processing reactions are posttranscriptional events and that polyadenylation precedes splicing (2, 9, 22). However, more recently it became clear that this is not a general pathway. Using electron microscopy, Beyer and Osheim (5) presented evidence that pre-mRNA splicing occurs cotranscriptionally on nascent transcripts of *Drosophila* embryo genes. In addition,

nascent transcripts from the *Drosophila E74A* gene are spliced before they are polyadenylated (18).

Our understanding of the precise mechanism and temporal order of RNA splicing and polyadenylation in trypanosomes is still limited. What has become clear is that pre-mRNAs from actively transcribed genes are processed rapidly, most likely cotranscriptionally, by *trans* splicing (this study) or polyadenylation (11). It will be interesting to determine whether this is a general rule for the processing of pre-mRNAs or whether the output of low-abundance mRNAs is achieved by regulating the efficiency of RNA-processing events.

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